

# **FANCIJ regulates the stability of FANCD2/FANCI proteins and protects them from proteasome and caspase-3 dependent degradation**

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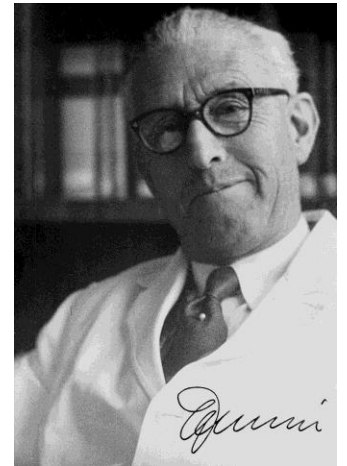
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Mitchell Cancer Institute  
University of South Alabama

# Outline

- Fanconi anemia (FA) pathway
- Role of FA pathway in Genome maintenance
- FANCD1 and FANCD2 functional relationship
- FANCD1-mediated DDR in response to Fork-stalling

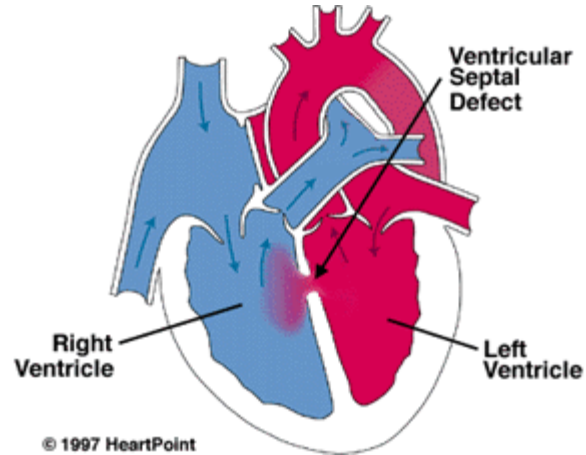
# Fanconi Anemia

- Rare, inherited blood disorder.
- 1:130,000 births
- Affects men and women equally.
- Affects all racial and ethnic groups
  - higher incidence in Ashkenazi Jews and Afrikaners



Guido Fanconi  
1892-1979

# Birth Defects

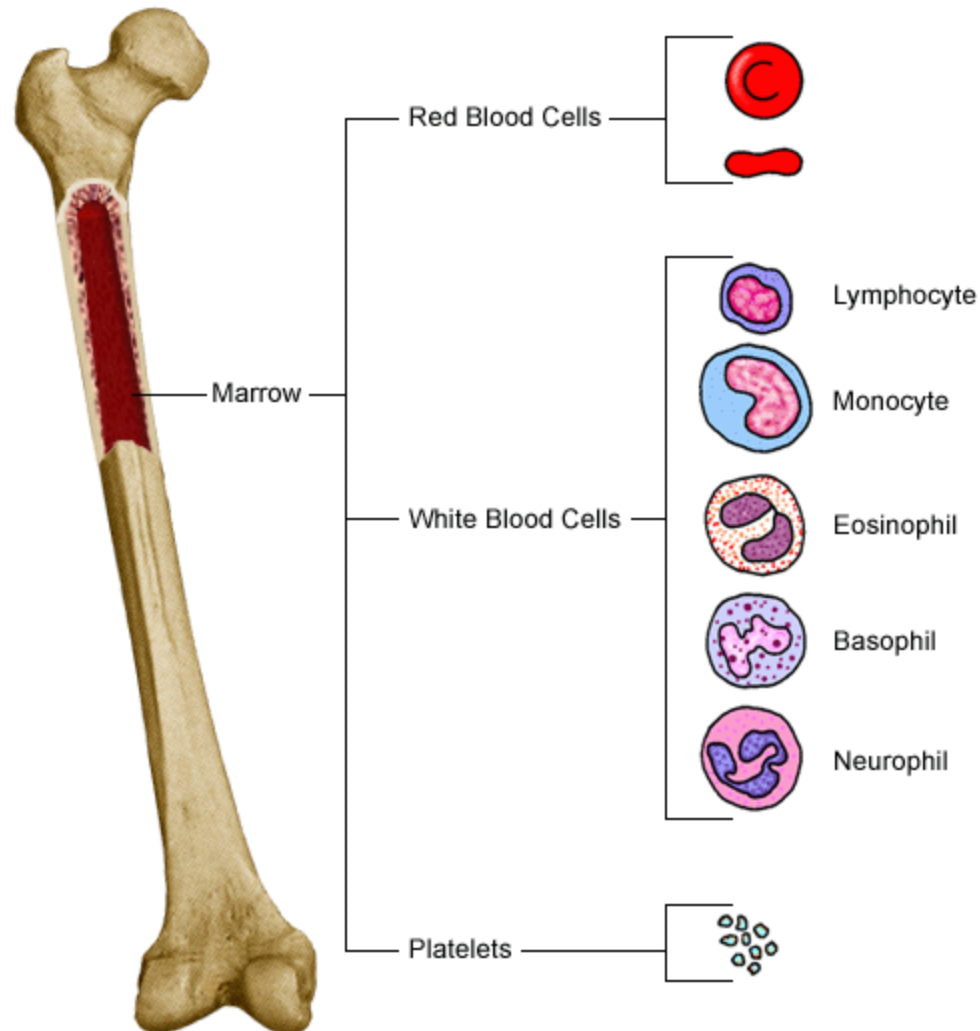


# Fanconi anemia pathway

- FA is a rare chromosome instability syndrome
- Autosomal recessive disorder (or X-linked)
- Developmental abnormalities
- 17 complementation groups identified to date
- FA pathway is involved in DNA repair
- Increased cancer susceptibility
  - many patients develop AML
  - in adults solid tumors



# Fanconi Anemia is an aplastic anemia



# FA patients are prone to multiple types of solid tumors

- Increased incidence and earlier onset cancers:  
oral cavity, GI and genital and reproductive tract  
head and neck  
breast  
esophagus  
skin  
liver  
brain

Why?

# FA is a DNA repair disorder

- FA caused by mutations in 17 genes:

FANCA

FANCF

FANCM

FANCB

FANCG/XRCC9

FANCN/PALB2

FANCC

FANCI

RAD51C/FANCO

FANCD1/BRCA2

FANCI

SLX4/FANCP

FANCD2

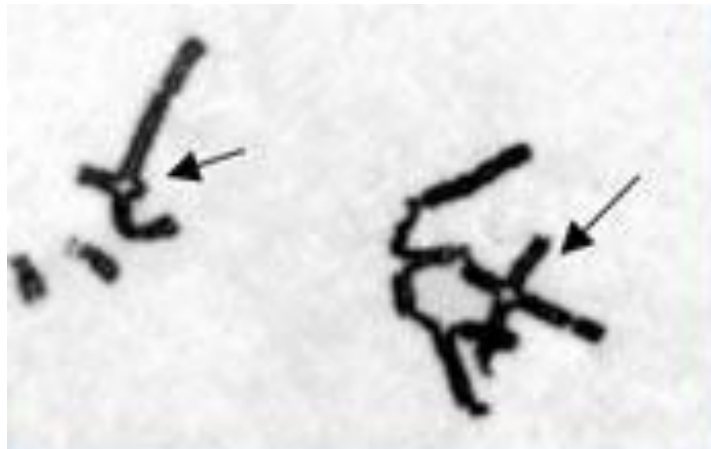
FANCL

ERCC2/XPF/FANCC

FANCE

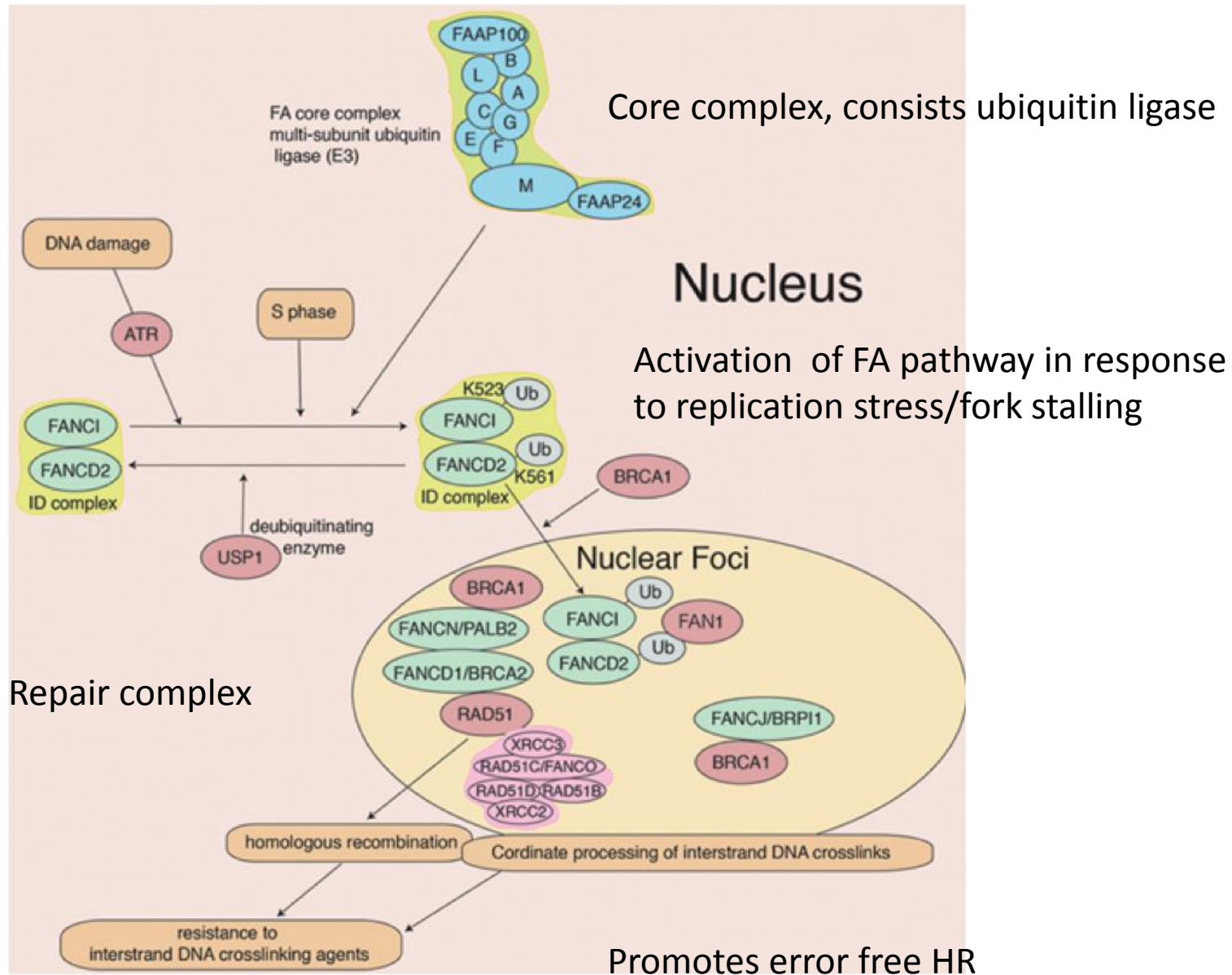
BRCA1/FANCS

- FA genes function in DNA repair processes
- FA patient cells are highly sensitive to DNA crosslinking agents





# Fanconi anemia is a disease of DNA repair

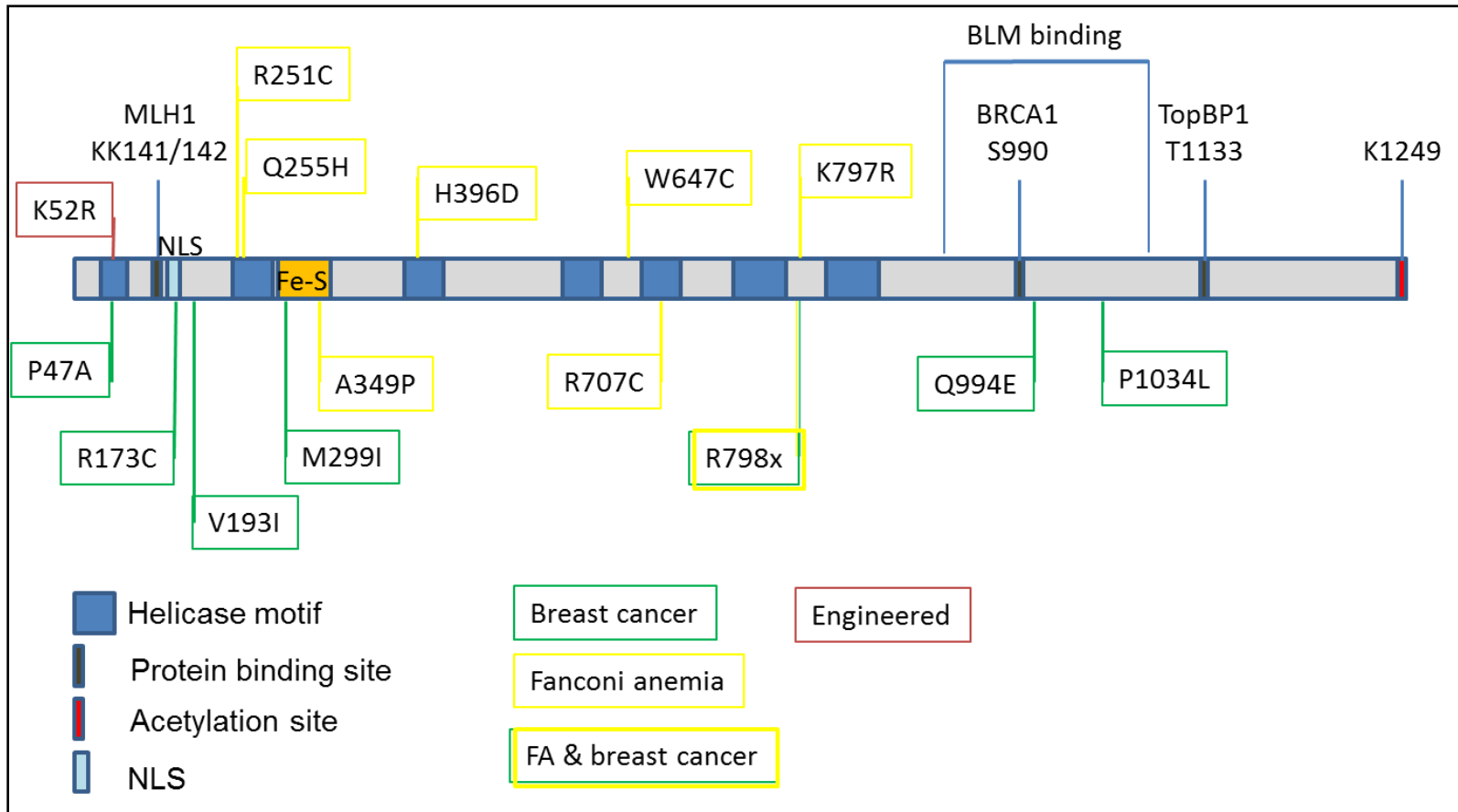


# FANCI

- 5' to 3' DNA helicase in RecQ family
- Acts as a tumor suppressor
- Directly associates with BRCA1
- Also known as BRCA1-interacting protein 1 (BRIP1)
- FANCI mutations lead to increased risk of breast and ovarian cancers
- FANCI known to act downstream to FANCD2 in DNA repair

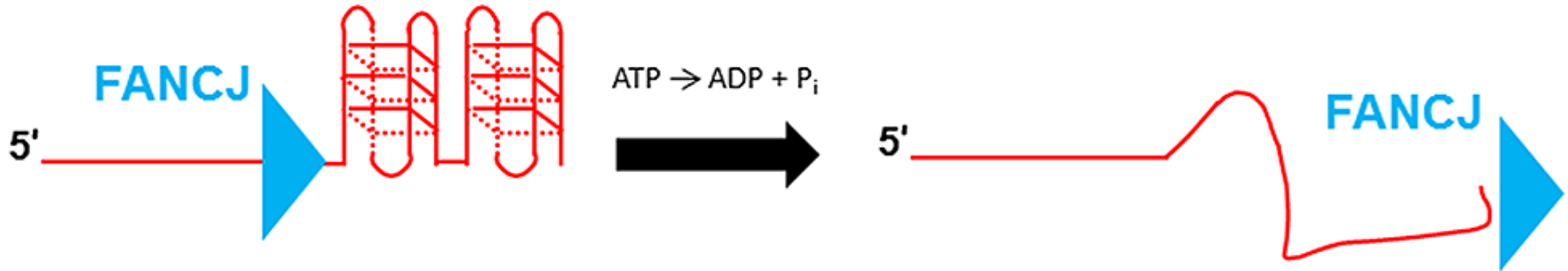
# FANCDJ interacts with numerous proteins that function in DNA damage response / repair

- Replication protein A (RPA)
- BRCA1 & 2
- BARD1
- RAD51
- MutL Homolog 1 (MLH1)
- Topoisomerase II binding protein 1 (TOPBP1)
- BLM
- FANCM
- The MRN complex – MRE11, RAD50 and NBS1



- Figure 15. Map of FANCI functional domains, protein interaction sites and mutations.** This figure illustrates the various domains of FANCI and the mutations. There are 14 clinically relevant FANCI mutants, including 7 identified in FA, 6 in breast cancer, and 1 in both. There are 3 mutations that are known to alter helicase activity – K52R abolishes activity and P47A and M299I decrease helicase function.

## G4 Resolution by FANCD Helicase



- Preservation of chromosomal structure
- Smooth replication
- Maintenance of genomic stability
- Transcriptional regulation

# FANCD2

- Considered the FA pathway effector protein
- Forms a complex with FANCI
- Monoubiquitinated by the FA core complex in response to DNA damage
- Activation of FANCD2 is necessary for repair of crosslinks and for homologous repair

# Evidence showing FANCD2 may act earlier in the pathway

Chromosoma (2010) 119:637–649

DOI 10.1007/s00412-010-0285-6

## RESEARCH ARTICLE

### FANCD2/BRIP1 recruitment and regulation of FANCD2 in DNA damage responses

Fan Zhang • Qiang Fan • Keqin Ren •  
Arleen D. Auerbach • Paul R. Andreassen

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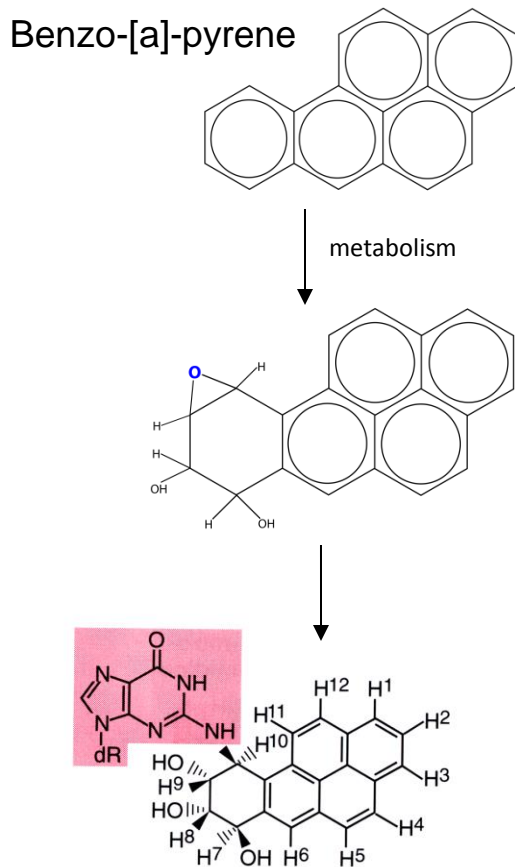
### The Fanconi Anemia Proteins FANCD2 and FANCD1 Interact and Regulate Each Other's Chromatin Localization\*

Received for publication, February 3, 2014, and in revised form, July 24, 2014. Published, JBC Papers in Press, July 28, 2014, DOI 10.1074/jbc.M114.552570.

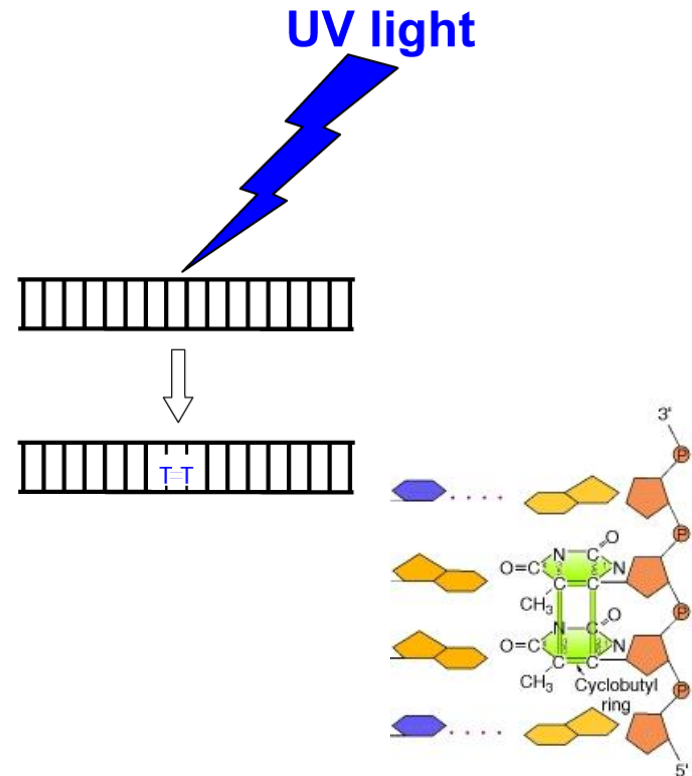
Xiaoyong Chen<sup>†1</sup>, James B. Wilson<sup>§1</sup>, Patricia McChesney<sup>‡</sup>, Stacy A. Williams<sup>‡</sup>, Youngho Kwon<sup>¶</sup>,  
Simonne Longerich<sup>¶</sup>, Andrew S. Marriott<sup>‡</sup>, Patrick Sung<sup>¶</sup>, Nigel J. Jones<sup>‡</sup>, and Gary M. Kupfer<sup>†\*§2</sup>

# Environmental Genotoxins

Poly aromatic hydrocarbons  
(PAH)

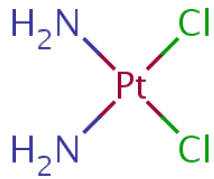


Solar UV radiation

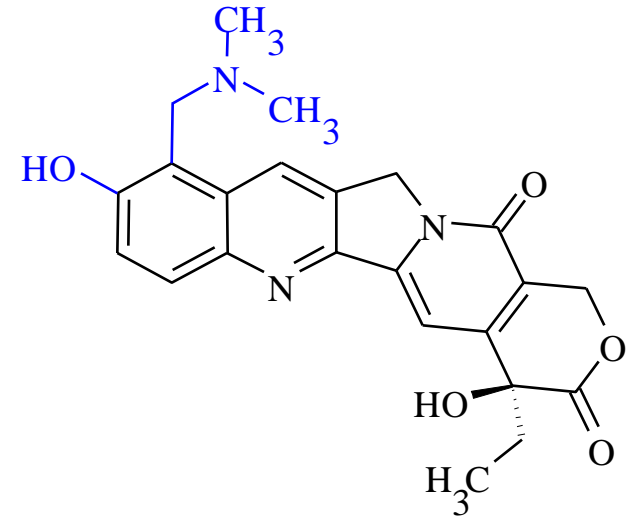




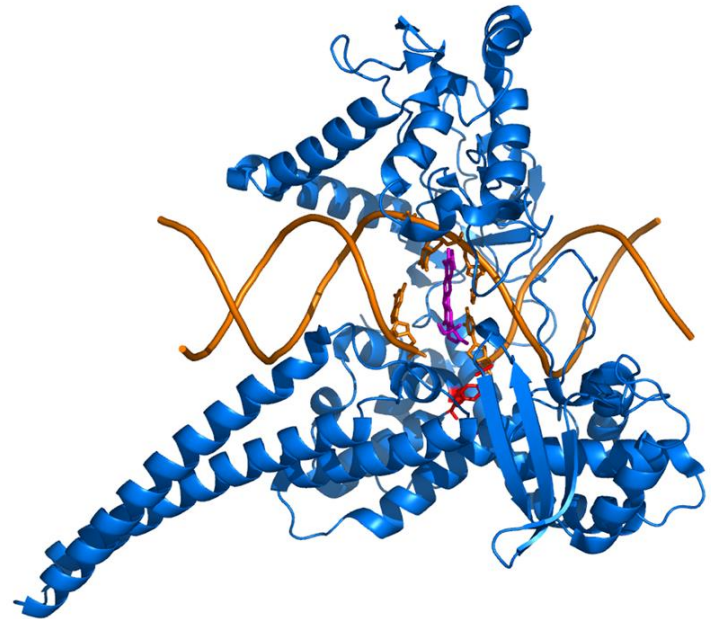
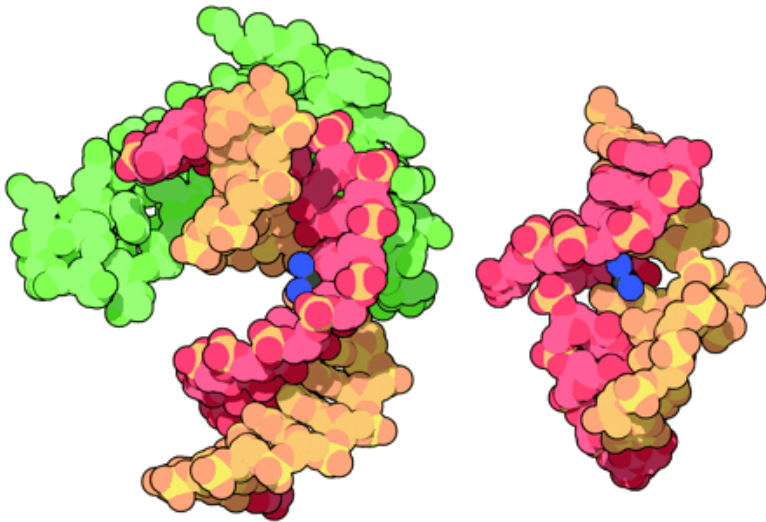
# Chemotherapeutic agents



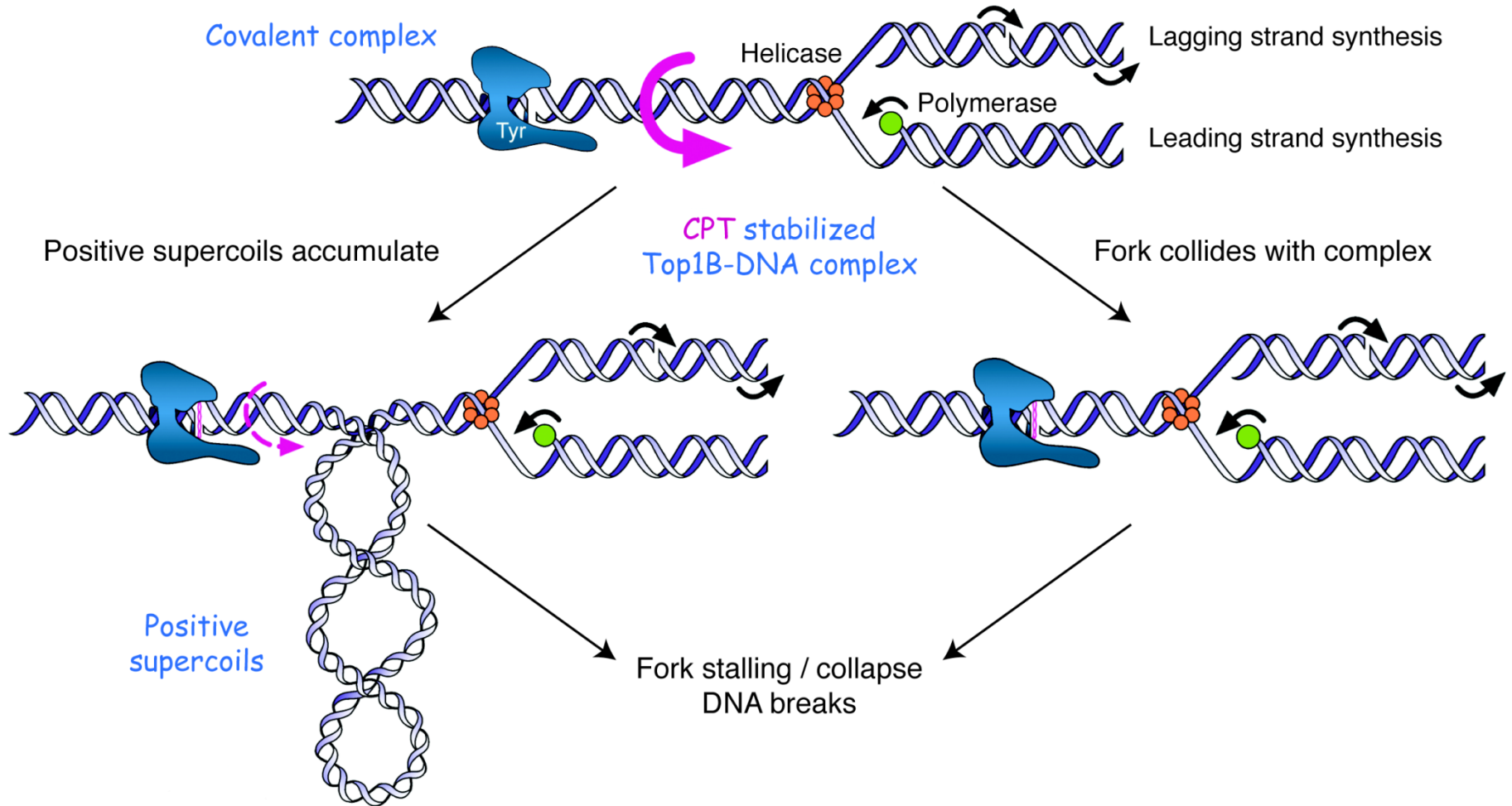
Cis-Platin



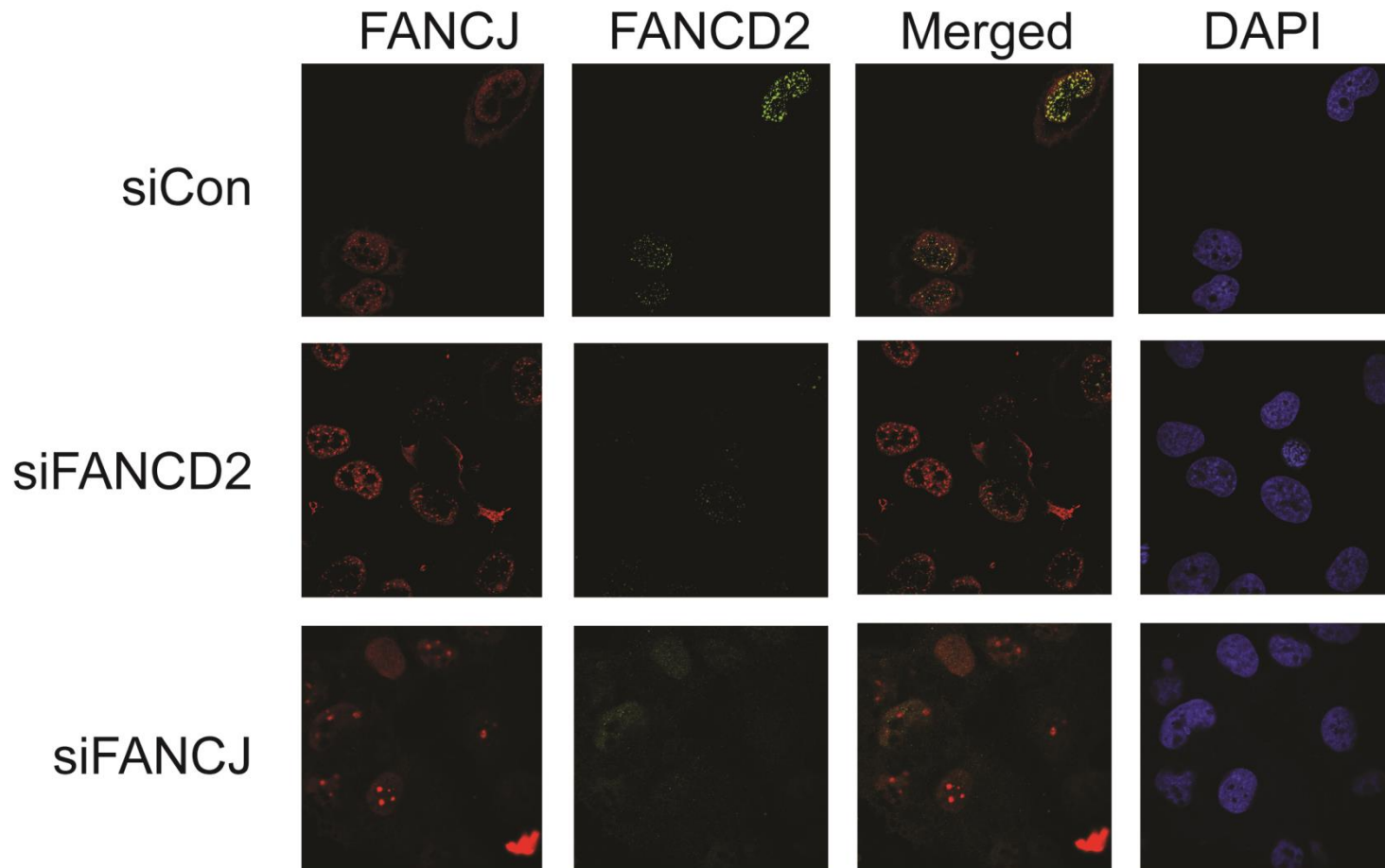
Camptothecin



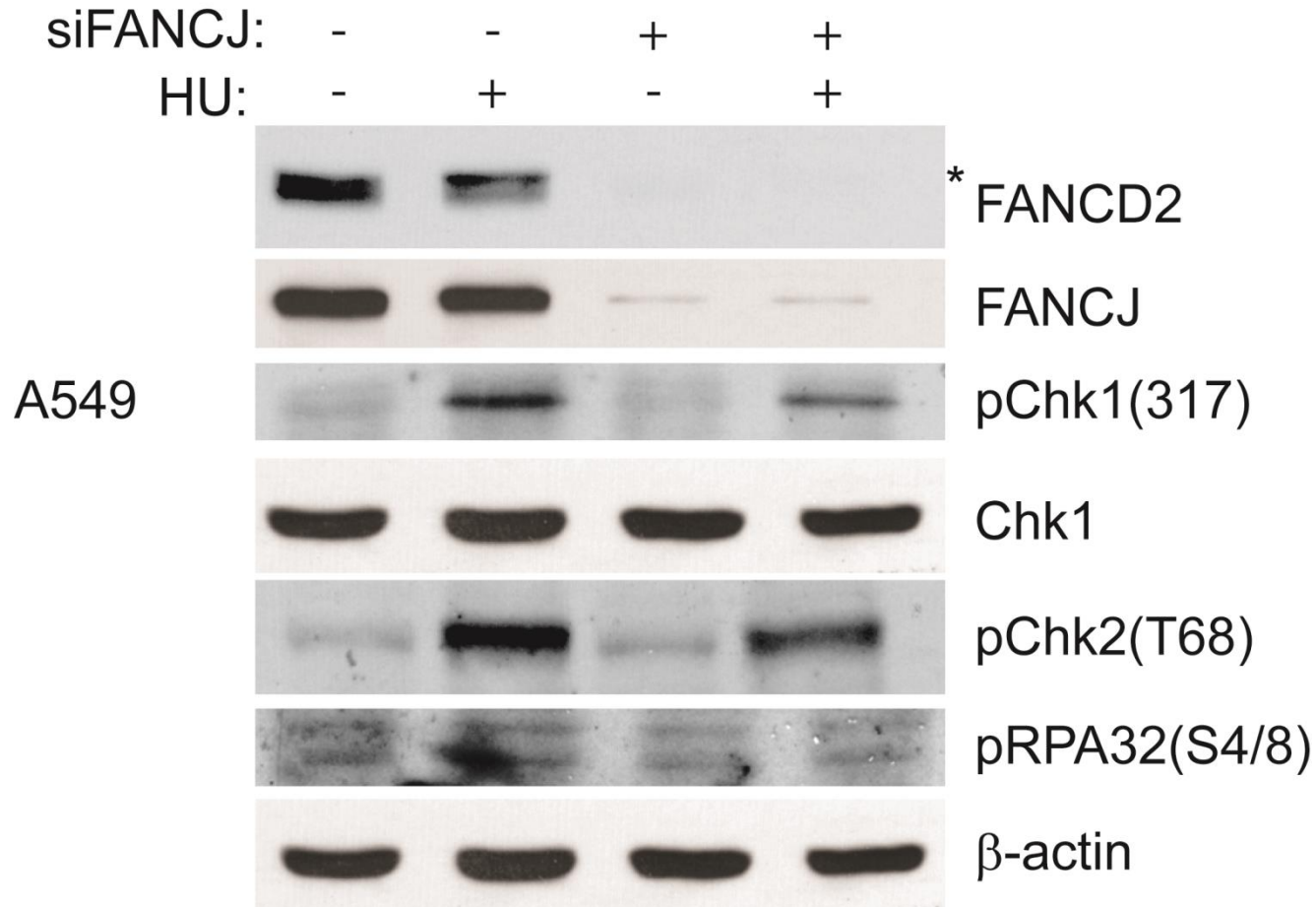
# Camptothecin poisoning of Top1 induces replication stress



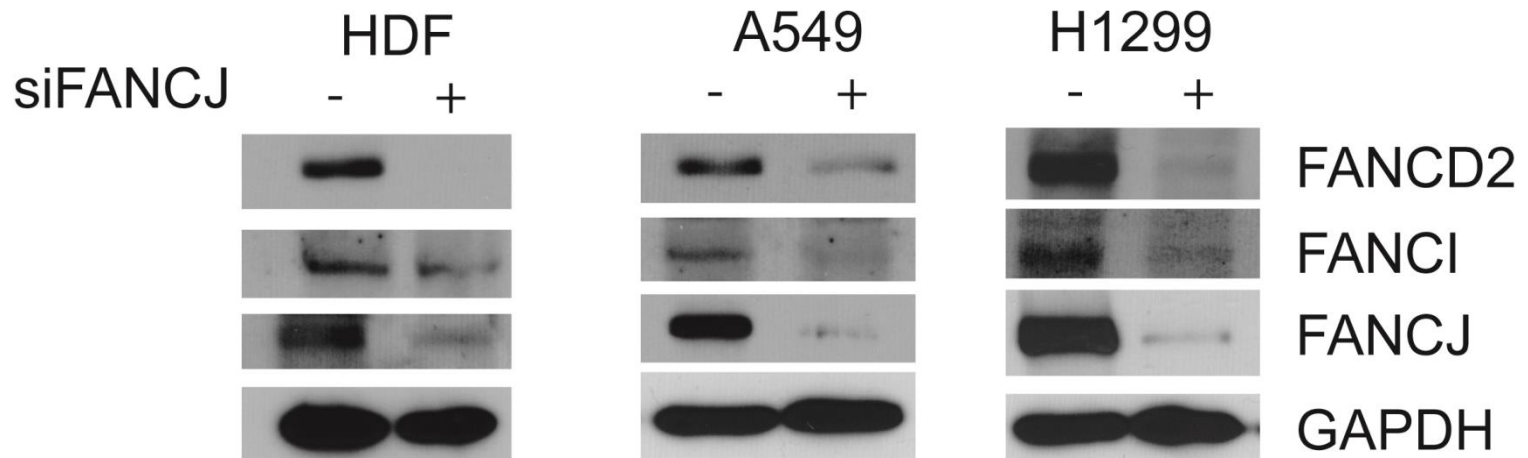
# FANCD2 is important for FANCD2 foci formation in response to replication stress



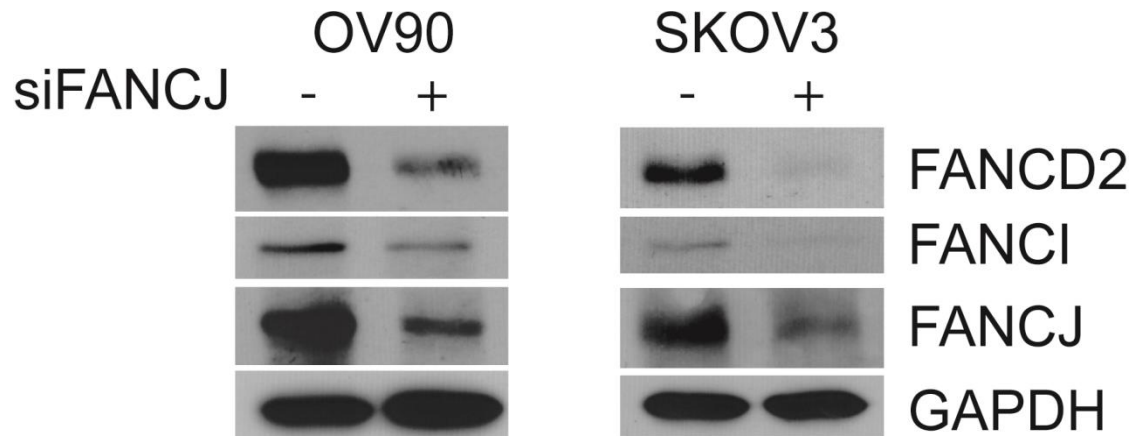
# FANCD2 is important for FANCD2 stability in response to replication stress



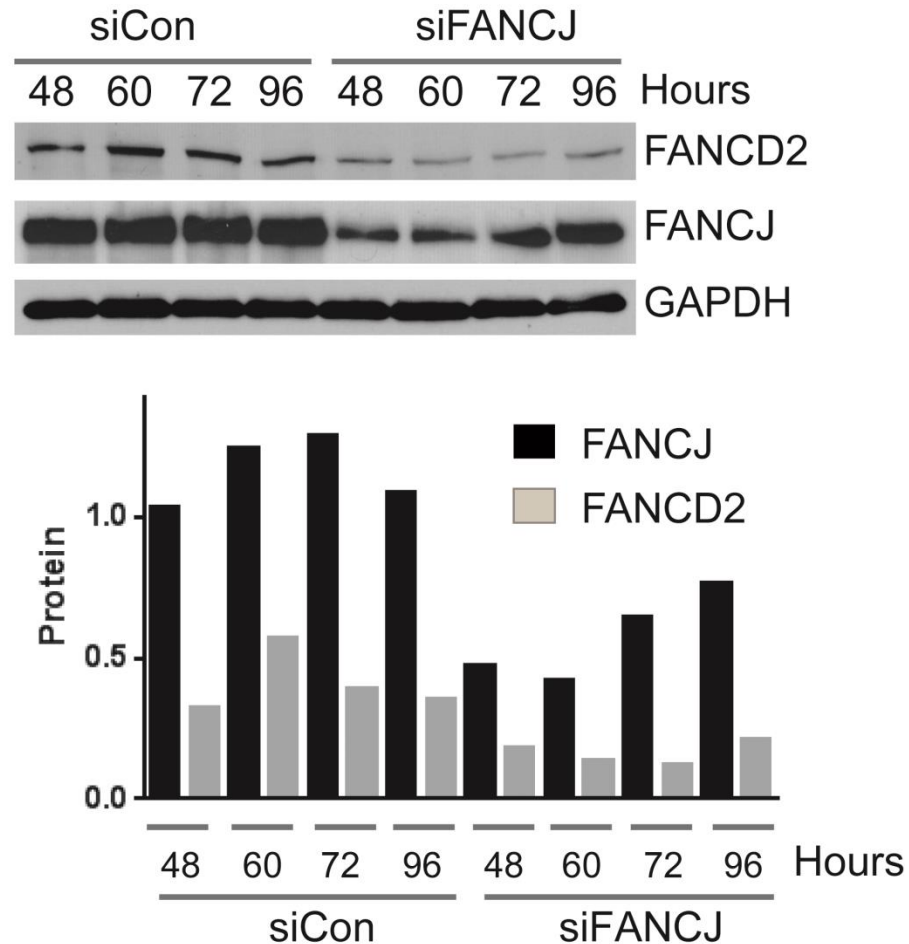
# FANCIJ regulates the stability of FANCD2/FANCI complex in multiple cell lines



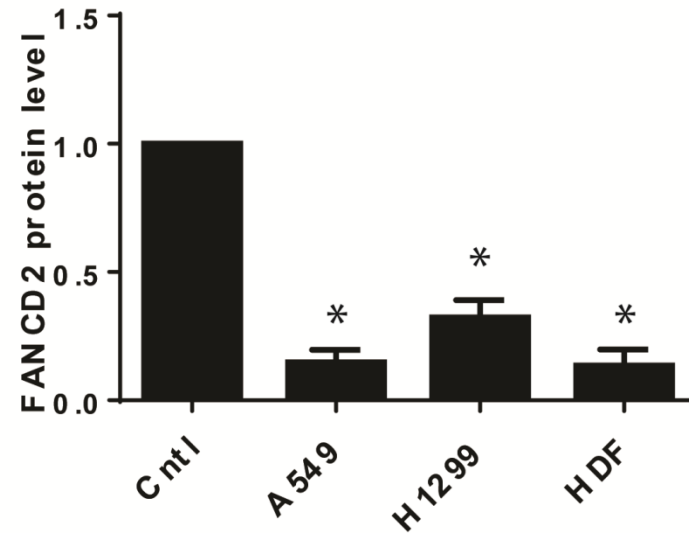
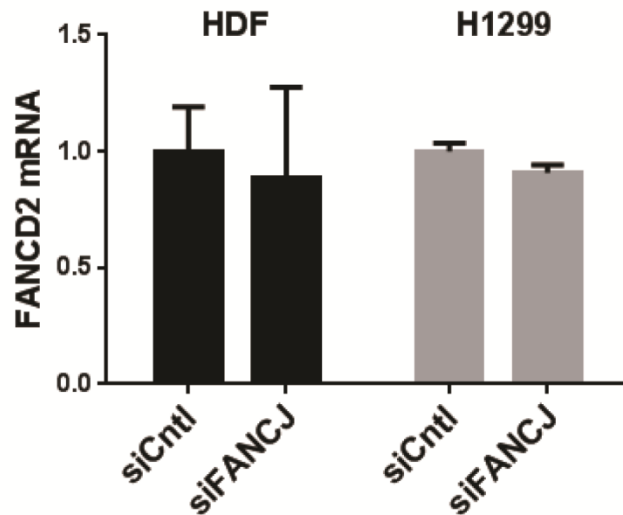
# FANCIJ regulates the stability of FANCD2/FANCI complex in multiple cell lines



# FANCD2 stability depends on FANCDJ protein

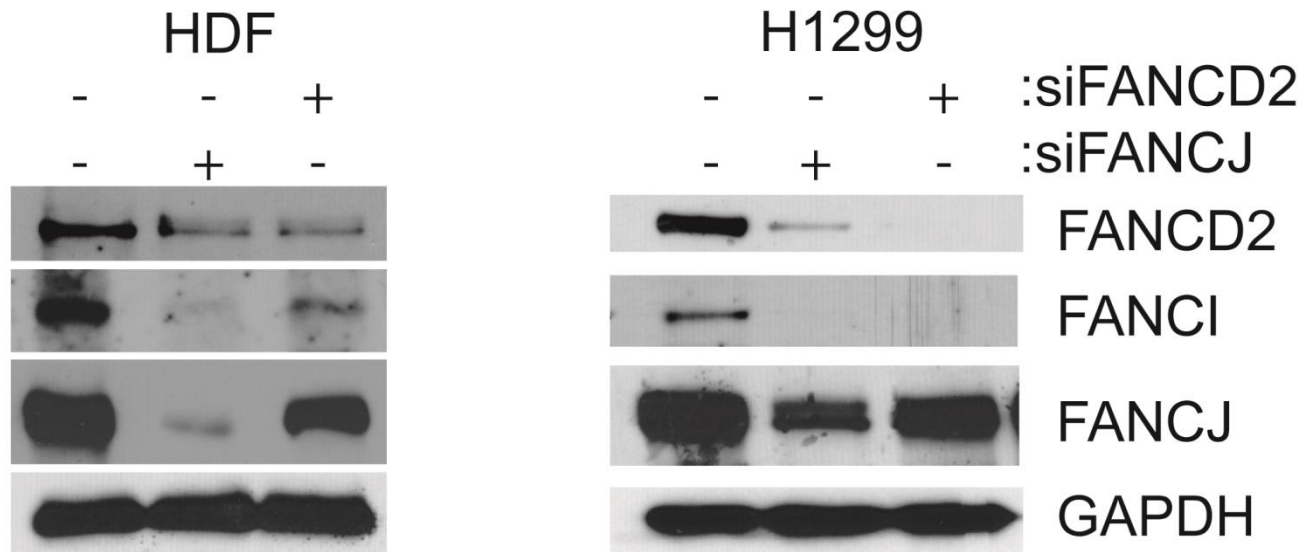


# FANCI regulates stability of FANCD2/FNACI proteins

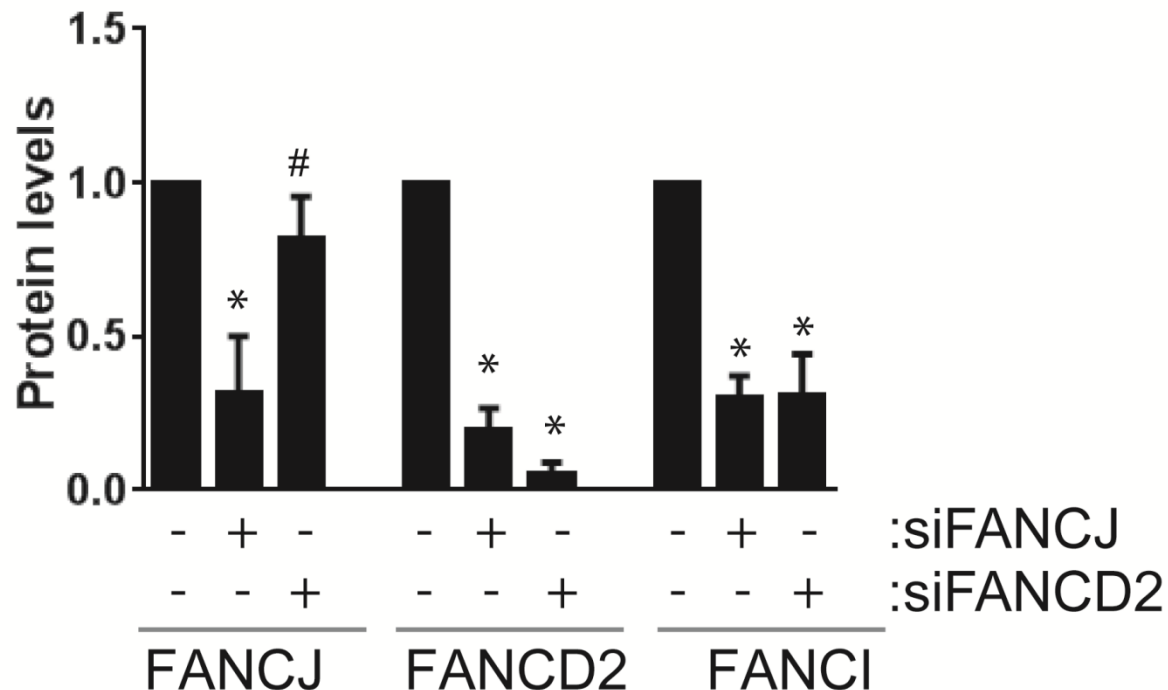




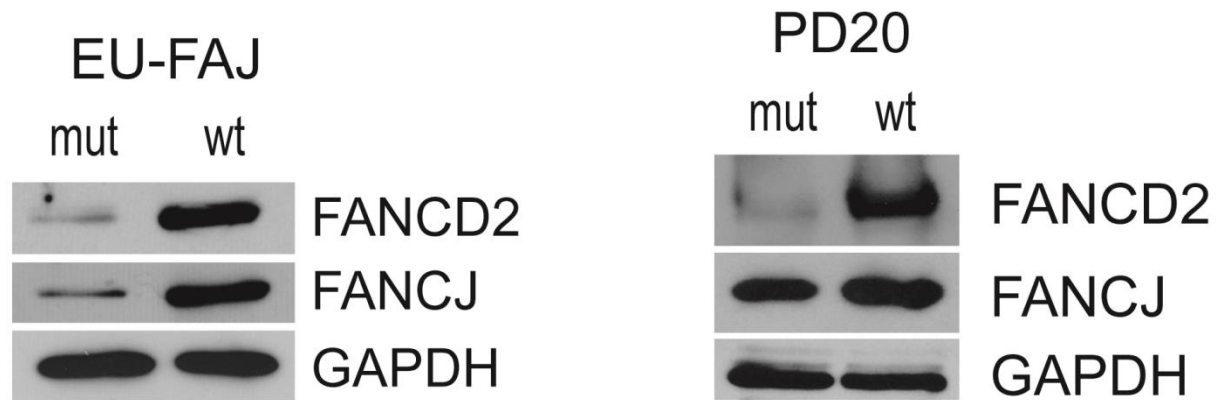
# FANCD2 status affects FANCI but does not influence much on FANCIJ protein



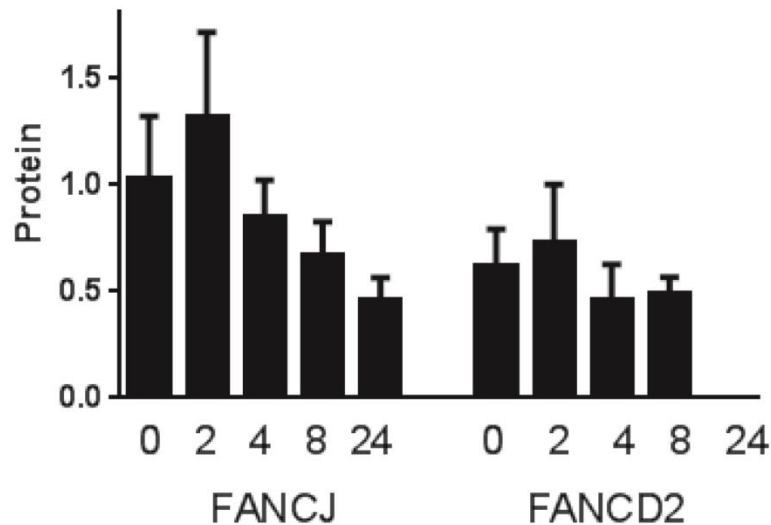
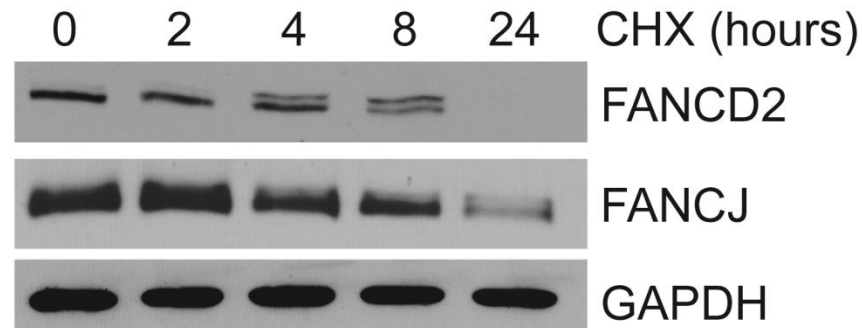
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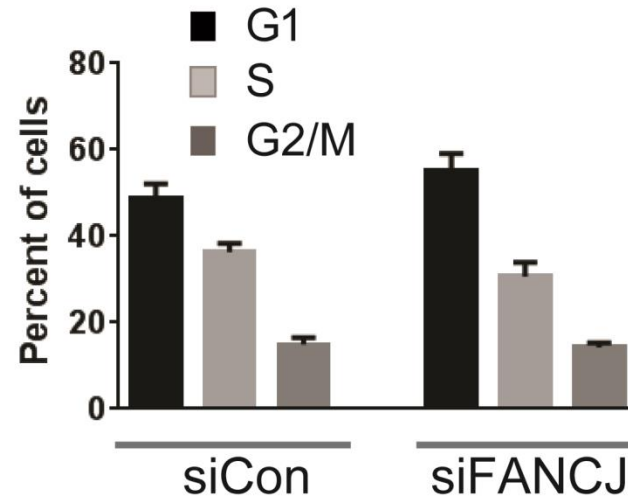
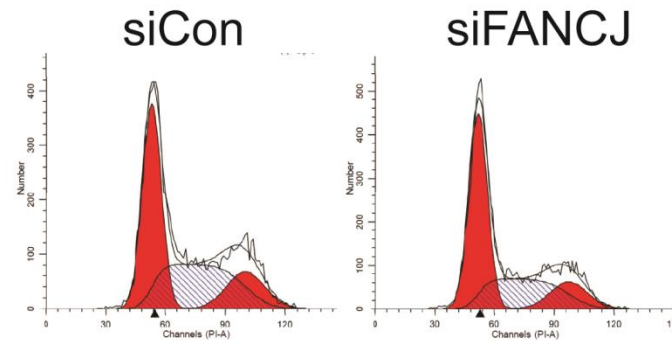
# FANCD2/FNAC1 complex



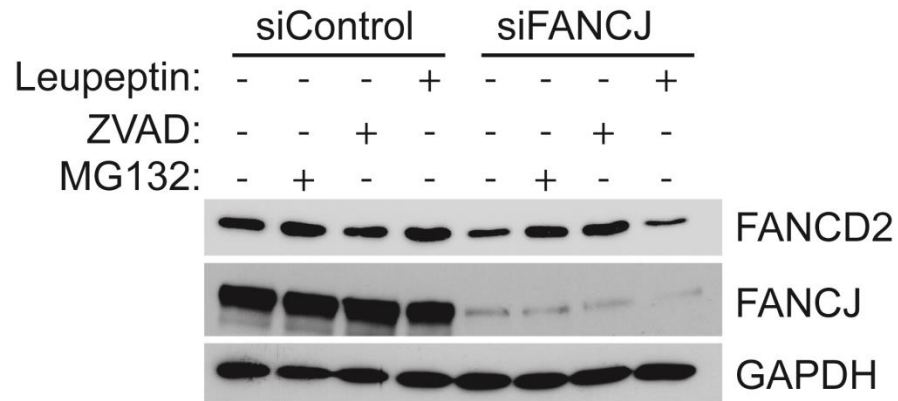
# Cyclohexamide treatment reveals FANCD2 protein levels depends on FANCD2



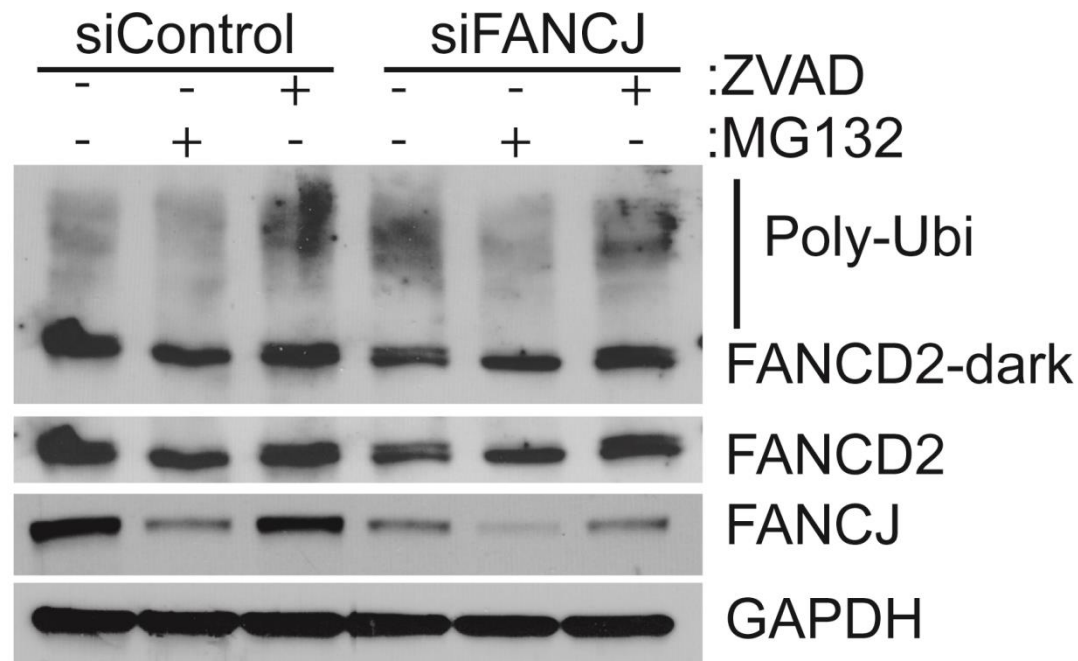
# FANCIJ deficiency does not affect cell cycle profile



# Proteasome and Pan-caspase inhibitors rescue FANCD2 protein in the absence of FANCD1



# Pan-caspase inhibitor rescue FANCD2 protein in the absence of FANCDJ



# FANCD2

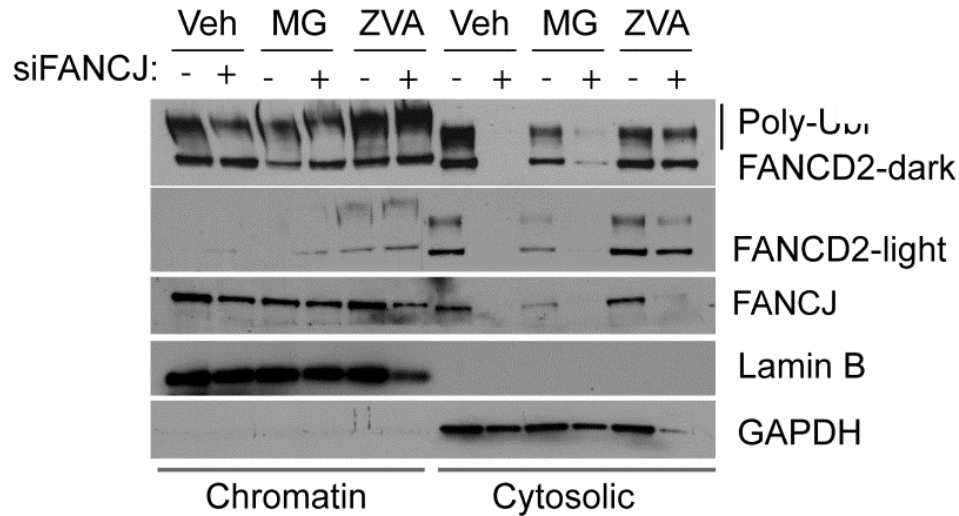
## FANCD2

FANCD2

# FANCD2

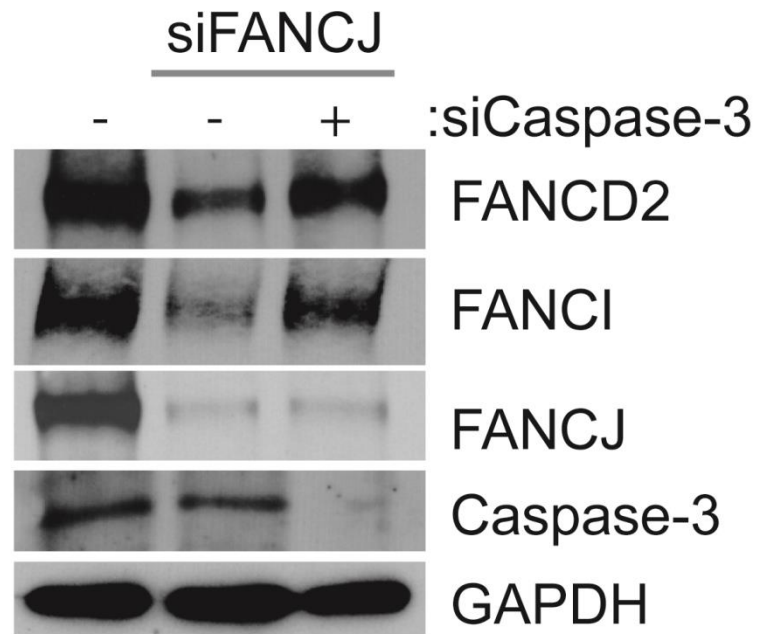
## FANCD2

FANCD2

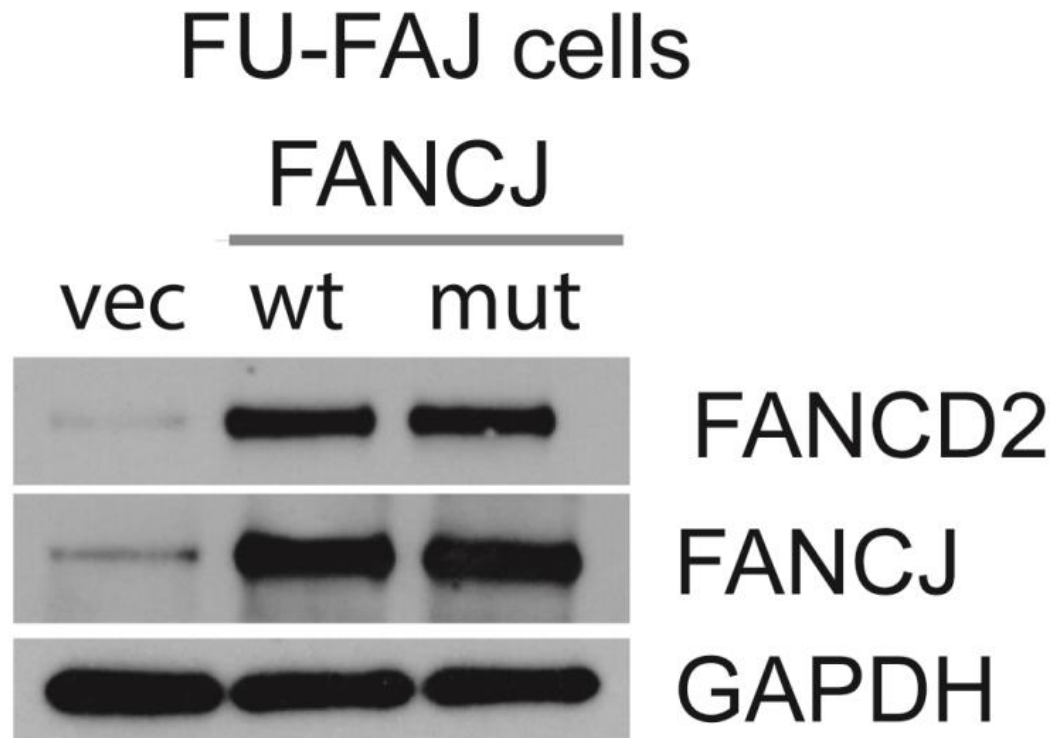




# Caspase-3 downregulation in FANCD2 deficient cells rescues FANCD2



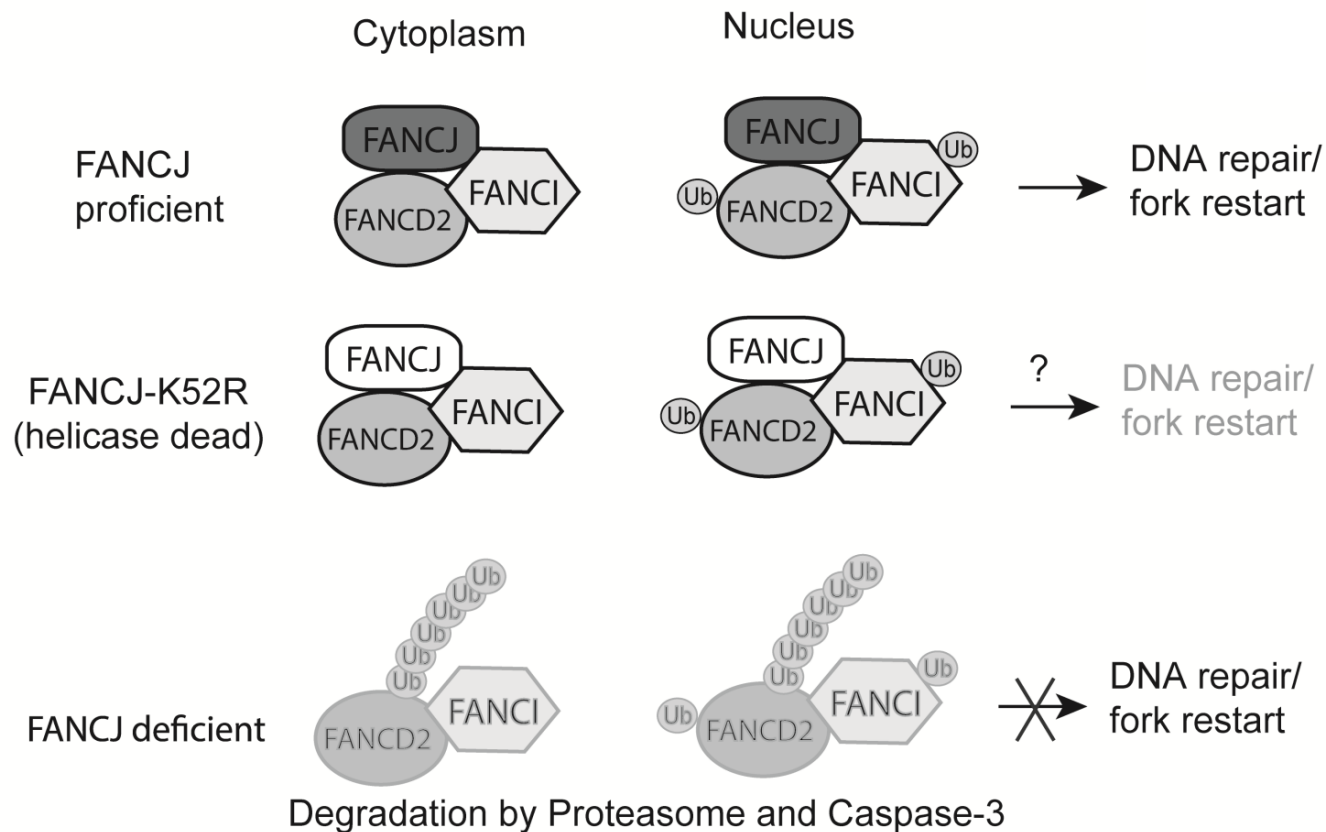
FANCD2 protein but not its helicase activity important for FANCD2 stability



# FANCI and FANCD2 proteins co-immunoprecipitates in the absence of DNA damage



# FANCI is important for integrity of FA pathway



# Summary

- FANCD2/FANCI complex is important for the stability of FANCD2/FANCI complex
- FANCD2/FANCI is necessary for proper activation of DNA damage response
- FANCD2/FANCI protects FANCD2/FANCI from degradation by the ubiquitin proteasome and caspase-3 dependent mechanisms
- FANCD2/FANCI is the key regulator of DDR to replication inhibitors
- FANCD2/FANCI has both helicase dependent and independent functions in DDR

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